THE BACTERIOLOGIC ANALYSIS OF THE FECAL FLORA OF CHILDREN

WITH NOTES ON THE CHANGES PRODUCED BY A CARBOHYDRATE DIET

G. B. MORRIS; R. L. PORTER, AND K. F. MEYER

From the George Williams Hooper Foundation for Medical Research, Pediatrics Department, University of California Medical School, San Francisco

Certain extremely characteristic symptoms in children from 3 months to 8 years of age have for many years attracted the attention of one of us. These symptoms could well be explained by the assumption of an existing abnormal intestinal flora, so that we undertook a systematic study of these metabolic disorders of suspected intestinal origin by careful quantitative and qualitative bacteriologic stool examinations. The purposes of these tests were twofold: first, to verify and support the contention of the clinician that a putrefactive flora is associated with a definite syndrome in which bacteriologic data may possibly be of diagnostic value; secondly, to establish, by means of a standard technic, certain indexes by which the various types of fecal floras could be readily recognized, whereby the transforming influence of certain foodstuffs could be controlled.

Unfortunately, a complete method of analysis for infants' stools, based on the recent knowledge of gastro-intestinal bacteriology, was not available. We have, therefore, in the last year adopted a set of procedures which has been of great service in the study of the above mentioned intestinal disorders, and these will be detailed in this communication.

The technical procedures employed today in the study of the fecal flora are the outcome of a definte evolutionary development of the field of intestinal bacteriology. It would be of interest to review these historical facts, but for the sake of brevity we refer in this connection to the monographic presentations of the subject in the publications of Schmidt,[1] Sittler,[2] Escherich,[3] Moro,[4] Kendall,[5] Torrey[6] and others.

Received for publication June 10, 1919.

[1] Die Fäzes der Menschen, 1910, p. 308.

[2] Die Wichtigsten Bakterientypen des Säuglingsdarmes, etc., 1909; Centralbl. f. Bakteriol., 1908, 47, pp. 14 and 145.

[3] Die Darmbakterien des Säuglings, 1886.

[4] Jahrb. f. Kinderh., 1905, 61, pp. 687, and 870.

[5] Bacteriology, General, Pathological, Intestinal, 1916, p. 579-600; Jour. Biol. Chem., 1909, 6, p. 499.

[6] Jour. Infect. Dis., 1915, 16, p. 72-108.

MATERIAL AND METHODS

As already stated, it was our aim to establish by means of certain culture mediums and by microscopic examinations a standard by which the biologic activities of the fecal flora of children as a whole could be recognized. A total of over 250 specimens of diseased, and 12 samples of perfectly healthy children have been examined. In a series of cases repeated bacteriologic analyses were made to control the influence of diet on the improvement of the clinical symptoms. The stool specimens were all obtained from cases which exhibited the symptoms of intestinal intoxication described in another publication, and were under the constant supervision of one of us (L. P.). Stool specimens of children treated for surgical diseases, in the Children's Department of the University of California Hospital, served as controls; these patients did not suffer from intestinal disturbances and received a liberal mixed diet.[7]

METHODS OF EXAMINATION

The technic employed in this study was chosen after a careful critical experimental comparison of the methods suggested by Kerr, MacNeal and Latzer[8] and those of Torrey. The methods described by the latter proved to be exceedingly valuable and were used in preference to the others. The various steps of the procedures finally chosen are presented in outline form in the paragraphs which follow. The selection of the cultural methods is by no means considered as final; in fact, we have added several improvements since we collected the data on which this paper is based. On the other hand, we hope that a description of a dependable technic will stimulate pediatrists to apply the same to the study of intestinal disorders of infants.

Collection of Stool Specimens.—Each stool was passed directly on a sterile piece of gauze and placed inside of a sterilized quart jar. Attempts were made to obtain specimens from the early morning movement. The samples were cultured in the next two or three hours after collection, immediately on their receipt in the laboratory. During the course of preparation for the analysis, the dilutions, etc., they were kept on ice.

TECHNIC FOR THE EXAMINATION OF A STOOL SPECIMEN

1. *Preparation of the "Stock Dilution":*
   (1) Weighing: weigh 500 mg. of stool on a sterile watch-glass.
   (2) Emulsifying: wash thoroughly into a sterile mortar with 10 c c of sterile salt solution; rub with pestle until a homogeneous emulsion, free from visible clumps, is obtained.
   (3) Dilution: transfer to a small Erlenmeyer flask and dilute with salt solution to a volume of 50 c c. Each c c of this "stock solution" represents an equivalent of 10 mg. of fecal material. Fluid stools are emulsified in salt solution and standardized to the same density as one of the known weighed preparations.

[7] The diet consisted of:

(a) Three-fourths formula:		
	Whole milk	600 c c.
	Water	200 c c.
	Dextro maltose 0.5 per cent. solution	24 c c.
	Barley water	11 c c.
(b) One-half formula:		
	Whole milk	600 c c.
	Water	600 c c.
	Dextri maltose 0.7 per cent. solution	59 c c.

[8] Jour. Infect. Dis., 1909, 6, pp. 123 and 571.

2. *Microscopic Examination and Differential Count:*
   (1) Prepare smear from the "stock dilution" and stain by Gram's method.[9]
   (2) With the aid of a squared field ocular make a direct count of at least two fields of the following:
      (a) Percentages of gram-negative organisms.
      (b) Percentages of gram-positive organisms.
      (c) Percentages of gram-positive rods.
      (d) Percentages of gram-positive cocci.
3. *Plating Procedures:*
   (1) "Dally" one standard loop of stock dilution over the surface of one or two carefully dried Endo plates (Robinson's and Rettger's modification).
   (2) Sugar-free veal or beef liver-infusion-Difco-peptone-agar plates; reaction p_H 7.0-7.2.
      (a) Prepare from the "stock dilution" the following dilutions: 1:1,000, 1:10,000, 1:100,000.
      (b) With 1 c c of the dilutions 1:10,000 and 1:100,000 prepare pour-plates.
      (c) Count plates after 24 hours aerobic incubation at 37 C.
   (3) Lactose agar plates (sugar-free-veal-infusion peptone—1 per cent. lactose agar).
      (a) Prepare pour-plates using 1 c c of the dilutions 1:10,000 and 1:100,000.
      (b) Count plates after 48 hours anaerobic incubation at 37 C.
   (4) Spore plate:
      (a) Heat in a Pasteur pipet a portion of the stock dilution at 80 C. for 10 minutes.
      (b) Make a pour-plate of lactose agar using one half of the heated suspension. Save the remainder for inoculation of a fermentation tube.
      (c) Incubate aerobically for 48 hours at 37 C.
4. *Seeding of the Fermentation Tubes:*
   (1) 0.5 c c lots of the "stock dilution" are each seeded into the following tubes and incubated aerobically at 37 C. for 24 hours:
      (a) 1 per cent. glucose sugar-free-veal-peptone broth fermentation tube.
      (b) 1 per cent. lactose sugar-free-veal-peptone broth fermentation tube.
      (c) 1 per cent. saccharose sugar-free-veal-peptone broth fermentation tube.
      (d) Bromcresol-purple-milk-fermentation tube.[10]
   (2) Heated material from procedure 3, part 4, is inoculated into a fermentation tube of milk to which sterile defibrinated blood has been previously added.
      (a) Incubate anaerobically at 37 C. for 48 hours.
5. *Inoculation with Undiluted Stool:*
   (1) One loopful of the undiluted fecal specimen is inoculated into the following mediums:

[9] Sterling's or carbol methylviolet 6B or BN one minute, Gram's iodin solution — one minute, decolorized with acetone-alcohol (1:3) and counterstain with dilute carbolfuchsin.

[10] Clark and Lubs: Jour. Agric. Res., 1917, 10, p. 105-111.

(a) Gelatin stab tube melting point 28 C.[11]
(b) Loeffler's serum slant medium.
(c) 1 per cent. lactose-peptone-ox-bile.
(2) Incubate (b) and (c) aerobically at 37 C. from 24 to 48 hours.
(3) Incubate (a) aerobically at 22 C. from 24 to 48 hours.

6. *Acetic Acid Glucose Broth Tubes:*
(1) 0.5 c c of the "stock dilution" is inoculated in the following tubes:
(a) N/5 acetic acid veal-infusion—1 per cent. glucose-peptone-broth tubes.
(b) N/10 acetic acid veal-infusion—1 per cent. glucose-peptone-broth tubes.
(c) N/20 acetic acid veal-infusion—1 per cent. glucose-peptone-broth tubes.

For anaerobic cultures the plates are placed in a Novy or museum jar, from which the air is exhausted and the vacuum replaced by hydrogen. The results noted in the various culture mediums are recorded over a time interval of from 24 to 72 hours; the growth in the fermentation tubes is expressed in percentage of gas and by symbols designating the degree of turbidity. Liquefaction and digestion of gelatin and Loeffler's serum are tabulated as follows: + slight; + + moderate; + + + rapid and heavy growth.

THE SIGNIFICANCE AND THE INTERPRETATION OF THE TESTS

The various culture mediums just described were chosen for the analyses of children's stools, because as a whole they produced a fairly true picture of the character of the stool flora and changes therein could be detected readily. For practical purposes it is reasonable to assume that the fecal flora represents the mirror picture of the flora of the lower portion of the intestinal tract; for accurate scientific work on the flora of the digestive tube, more extensive studies on necropsies of children are necessary before the findings and deductions derived on laboratory animals can be accepted as identical with those assumed in human beings. (See Sittler, 24, page 15.)

We distinguish three types of intestinal floras: The fermentative or saccharolytic; the facultative or normal; and the putrefactive or proteolytic type. Each of these is characterized by a certain definite group of bacteria which on culture mediums hold true to species and to type, and their biochemical activities produce certain end results which enable the bacteriologist to recognize the type of flora.

The obligate flora of the children's stool is unlike that of an adult; the cultural results of an infant's stool vary from the findings in adults that have been recorded by some of the writers mentioned in the introduction. These differences therefore need a detailed discussion which can well be incorporated in a consideration of the purpose of each bacteriologic test.

[11] For method of preparation see: Forster: Centralbl. f. Bakteriol., 1897, 22, p. 341.

(1). *Microscopic Examination and Differential Count of the Direct Smears Made from the Feces and Stained by Gram's Method.*—Little need be said concerning the results obtained in the examination of the smears prepared from the "stock dilutions." In our experience we found that cultural procedures gave more reliable information with regard to the bacterial flora of the digestive tube. This conclusion is quite in accord with the statements made by Kerr, Latzer and MacNeal. Even the differential count can be very misleading and we hope that this abbreviated, antiquated form of stool analysis will ultimately be discarded as one of the routine procedures in the physician's office. On the other hand, a gram-stained stool smear is a considerable advance over nothing. It is not uncommon to find the pediatrist preparing slides with fat and starch stains, but he fails to investigate, even microscopically, the flora of the suspended fecal specimen.

(2). *Fermentation Tubes.*—Herter and Kendall[12] have both advocated the use of fermentation tubes containing sugar broths as important aids in a stool examination. The fact that these tubes offer an environment with varying oxygen tensions enables certain bacteria, usually present in the feces in very small numbers, to develop vigorously. According to the workers mentioned in the foregoing, the bacterial growth in a fermentation tube represents closely the viable microorganisms present in the stool. The volume of gas produced is, according to Kendall, of some diagnostic value. Under normal conditions of the digestive tube, closely similar amounts of gas are formed in the glucose, lactose and saccharose tubes. He also found that in adult cases suffering from intestinal disorders the percentage of gas usually increased above normal. On the other hand, in fermentative stools in which the aciduric organisms predominated, gas production was diminished. These general conclusions have not been confirmed through the studies of MacNeal, Latzer and Kerr, and Torrey. The amount of gas produced varied in the stool specimens of several normal individuals repeatedly examined by the first mentioned writers.

Smears prepared from the sediments of the different fermentation tubes and stained by Gram's method gave some insight into the occurrence of the various fecal bacteria. It may be said, however, that the relative distribution of the various organisms present was never clearly indicated inasmuch as the carbohydrate substances influence one or the other type to its advantage over the obligate flora. For

[12] Jour. Biol. Chem., 1909-10, 7, p. 203.

example, the saccharose fermentation tube always favored the development of streptococci and even when the presence of B. bifidus was otherwise demonstrated, this organism was suppressed and entirely absent in this particular medium. And again, the same stool specimen inoculated in lactose tubes would show a predominance of B. bifidus and the acidophilic bacilli with a suppression of the streptococci to a noteworthy degree. The lactose fermentation tube was therefore particularly valuable because it demonstrated the main flora of the infants' stool; the three characteristic pleomorphic forms of B. bifidus, so ably described by Herter in his book, were always readily recognized.

As a whole, the percentage of gas production was not so characteristic as it has been described for fecal specimens of adults. In interpretating this difference we offer the following explanation: An excess of lactic and acetic acid evolved by B. bifidus reduces the H-ion concentration of the medium to such a degree that the growth of the ordinary gas producing bacteria, like B. coli and B. lactis aerogenes, is partially or completely suppressed, and therefore no production of CO_2 takes place. In our experience this absence or low percentage of gas in the glucose and saccharose tubes is very characteristic for normal infant stools. In the lactose tube, however, the extensive growth of B. bifidus, as is to be expected, produced a certain percentage of gas which was always higher than in the other two carbohydrate tubes employed. The growth of streptococci and enterococci caused intensive turbidity with little or no gas production in the saccharose fermentation tubes. In our experience this phenomenon was particularly noticeable when dealing with highly putrefactive stools.

In four examinations after 48 hours' incubation the open arm of the fermentation tubes turned to a brilliant green when kept at room temperature and exposed to light, denoting the presence of B. pyocyaneus. Sugar tubes seeded with fermentative stool specimens frequently developed a thick pellicle consisting of an extensive growth of yeasts. These tubes also liberated the odor characteristic of these organisms.

Lactose-peptone-oxbile medium enriches the organisms of the B. coli group and the aerobic and anaerobic spore bearers. Again, the gas production was not characteristic, but the smears from the sediments frequently enabled us to make an early diagnosis of the nature of the stool. Briefly, the microscopic findings of the various sediments may

be as follows: in a putrefactive, proteolyzing flora the smears show few aciduric organisms, numerous B. coli, gram-positive spore-bearing rods, and streptococci; in a fermentative flora aciduric organisms predominate, and very few B. coli are noted.

The percentage of gas collecting in the closed arms was always higher than in the above discussed plain broth-carbohydrate tubes This may in part be the result of a heavier seeding or the outcome of a restriction of the aciduric organisms which, as we have explained above, depresses the growth of the gas producers. Sometimes the percentage of gas offered suggestions for a possible diagnosis of the fecal flora, namely, when more than 60 per cent. of gas had collected in the closed arm. In these instances the specimen invariably contained a large number of B. welchii, as could be checked by the stormy fermentation which took place in the blood milk tubes. The maximum gas production as a rule was only recorded after 48 hours; the bacterial lag was probably affected by the alkaline reaction of the bile, which inhibited to a certain degree the biochemical activities of the inoculated bacteria.

The milk-fermentation tubes containing bromcresol purple[10] as a delicate indicator have proven in our hands to be of extreme value. Irrespective of the type of flora, whether putrefactive or fermentative, the reaction of the inoculated and incubated tubes was acid; the aciduric and B. coli organisms are naturally responsible for this result. Therefore, it is not the reaction but its constant occurrence that gives the character of the curd diagnostic importance. Stormy fermentation with maximum whey production was only recorded when B. welchii was present. A soft, yellowish curd with few gas bubbles and little or no whey was found to be produced by a fecal specimen containing primarily proteolytic bacteria. Such a curd on further incubation showed progressive peptonization and intensification of the yellow color. Organisms found in fermentative stools produce another very important reaction in the milk: The curd is firm, massive, with considerable whey; the closed arm may contain from 20 to 30 per cent. of gas. Frequently lenticular gas bubbles are seen in the otherwise smooth curd. Peptonization or changes in the color never take place, even after prolonged incubation. The descriptions of the two kinds of curds apply naturally only to the absolutely fixed types of a putrefactive or a fermentative flora. We have frequently noted, however,

less significant changes and have found it difficult to interpret these "pseudo reactions" when examined independently of the other mediums employed.

(3). *Loeffler's Serum Medium Slant and Gelatin Stab Culture.* — These two mediums supply a protein rich substratum and therefore favor organisms commonly encountered in putrefactive stool specimens. The degree of liquefaction and digestion of the respective mediums may well serve as a criterion for the number of viable putrefactive bacteria. In our experience the Loeffler's tube is an excellent diagnostic aid in stool analyses. Incubated for from 24 to 72 hours, the coagulated serum may undergo the following changes: either small lenticular and irregular depressions, so-called "biting in," accompanied by slight discoloration or complete digestion with intensive blackening of the dissolved serum and a strong putrid odor always proved the existence of a putrefactive stool sample. The organisms most frequently found in such peptonized tubes were bacteria of the B. coli group, streptococci and gram-positive sporulating rods. Detailed systematic studies still in progress have convinced us that the aerobic and anaerobic gram-positive spore-bearing rods in all probability constitute some of the constant elements of a putrefactive stool specimen of children. Slight growth, absence of liquefaction or changes in color and odor were associated with fecal bacteria of strictly fermentative specimens.

Gelatin stab cultures showed liquefaction of varying degrees, depending entirely on the source of the sample examined. Pronounced putrefactive feces caused complete liquefaction in 72 hours. In our series of observations three types were commonly recorded: (*a*) liquefaction along the needle tract with arborescent spreading into the depth of the medium; (*b*) liquefaction beginning about one-half to one inch below the surface of the gelatin column with little extension into the depth. This type indicates the presence of organisms with a delicately adjusted requirement for oxygen; (*c*) infundibuliform surface liquefaction progressing downward, with amber-brownish discoloration of the dissolved medium, suggests aerobic gram-positive spore bearers.

Until we have completed our study of the predominant organisms found in a putrefactive stool, it appears to us premature to interpret these types of liquefaction. It is not unlikely that certain clinical intoxications of gastro-intestinal origin are associated with a definite

group of proteolytic organisms; and therefore the types of gelatin liquefactions just described may have considerable diagnostic value.

The bacteria commonly found in a fermentative stool specimen changed the heavily seeded gelatin column and the Loeffler's serum slant in a few instances only. The microscopic examination of these cases demonstrated a large number of gram-positive spore bearers. Most of the specimens which gave this irregular reaction were derived from cases recently placed on a carbohydrate diet.

(4). *Plate Counts on Sugar-Free Agar Aerobically, and on Lactose-Agar Anaerobically.*—The counts obtained and calculated represent the number of viable micro-organisms per one milligram of fecal matter. The sugar-free, as well as the lactose-agar plates supplied some information concerning the relative distribution of the various important stool bacteria. In sugar-free-agar plates the majority of the non-aciduric organisms develop, as for example: B. coli, streptococci, etc. On the other hand, the very important aciduric bacteria multiply only in a medium containing carbohydrates, particularly lactose, and in an environment with reduced oxygen tension. When all the growth enhancing factors are fulfilled, these bacteria thrive satisfactorily in colonies, and a plate count represents the true number of viable facultative aciduric organisms. The following criteria served as aids in the recognition of the aciduric bacteria, B. bifidus and B. acidophilus; namely, marked pleomorphism of the gram-positive, fine rods, lenticular gas bubbles surrounding the surface colonies, and pronounced acetic odor of the plates. B. bifidus as a rule forms an oval or round colony with peripheral gas bubbles, while B. acidophilus is characterized by a cottony, fluffy, tuftlike center colony which is frequently surrounded by a dense turbid ring of sister colonies, giving a peculiar clouding of the medium.

The interpretation of the various counts obtained on the lactose-agar plates was sometimes difficult. As is well known, infants' stools always contain B. bifidus and B. acidophilus even in the most marked putrefactive stools. Therefore, the mere presence of aciduric colonies on the plates (in the ratio of 1:1) was not a criterion of a fermentative character of the specimen. Only when the ratio of the aciduric organisms is in a proportion of 2:1, is the flora certainly fermentative.

(5). *Endo Plates.*—In our hands this medium proved to be invaluable because an early diagnosis of the nature of the fecal material could be given. This medium, properly inoculated, will supply, even

on casual examination, immediate striking information. Putrefactive stools produce a heavy growth consisting of a variety of different types of colonies. A large spreading surface-growth covering the entire plate surrounds small groups of B. coli and possibly streptococci. The reaction of the medium is usually strongly alkaline, a red discoloration being noted only at the edges of the agar disk. This type of plate also gives forth a sweetish, pungent, musty odor. Quite in contrast to these findings are those recorded when a fermentative stool specimen has been plated. Few colonies, invariably those of B. coli, with their typical deep red color spreading throughout the medium, produce a marked acid odor. Similar results are obtained on bromcresol purple plates.

(6). *Spore Bearing Bacteria in Lactose-Agar Plates and Milk-Blood Tubes.*—Lactose-agar plates poured with the heated "stock dilution" specimen demonstrate the presence of aerobic spore-bearing bacteria. As a rule, growth was only noticed after from 48 to 72 hours' incubation. Gram-positive spore-bearing bacteria were regularly found in putrefactive stools; numerous and repeated examinations of stools recognized by other methods to be fermentative failed to demonstrate this group of organisms.

Fermentation tubes containing milk and, as an enrichment substance, blood, were always inoculated with the heated stool dilutions. Stormy fermentation with the findings of characteristic nonmotile gram-positive rods proved to be an indication of the presence of B. welchii or closely allied bacteria. In our studies we failed to find this organism in fermentative stools; putrefactive specimens, however, contained this bacterium frequently in large numbers. According to Herter,[13] B. welchii is considered to be one of the most important factors of intestinal putrefaction, being responsible for the so-called "saccharobutyric type" of gastro-intestinal intoxication. We are not as yet prepared to regard the B. welchii as one of the main causative organisms of intestinal proteolysis, but we are impressed with the absence of this organism in stool specimens of children kept on a high calory carbohydrate diet.

(7). *Acetic Acid Glucose Broth Tubes.*—In strongly putrefactive stool specimens the absence of aciduric bacilli is regularly shown by sterile acetic acid tubes. Marked growth in N/10 and N/5 acetic acid broth is therefore significant for a fermentative fecal flora.

[13] Bacterial Infections of the Digestive Tract, New York, 1907.

THE MAIN CHARACTERISTICS OF A NORMAL, A PUTREFACTIVE AND A FERMENTATIVE STOOL

From the above description of the methods and the interpretation of the findings, it is quite apparent that the three main types of fecal floras observed in children readily can be diagnosed with a great deal of accuracy. We summarized the results of about 150 different stool specimens in table 1. The data therein recorded represent the basic criteria by which a stool should be diagnosed as normal or as putrefactive or as fermentative.

Before we enter into a discussion of the bacteriologic differences between a normal, a putrefactive and a fermentative fecal flora, it is appropriate to say a few words concerning the macroscopic appearance of the stool samples. As a rule, the color and consistency of the specimen does not suggest the type of flora present except in fermentative types. A light buff-colored, foamy, semiformed stool specimen with a sour odor and strongly acid reaction to litmus or other indicators, is very suggestive of a fermentative fecal flora. On the other hand, proteolytic or putrefactive floras have been noted in fecal samples that can be grouped in the following three types: (1) dry, formed, solid dark-brown stools; (2) light colored, moist, mucus-containing stools with considerable undigested food debris accompanied by an offensive, putrid odor; and (3) samples of dirty, greenish color of semiformed, coarse consistency. The stool specimens of normal children have no characteristic macroscopic appearance, and vary according to the diet.

Returning to the discussion of the cultural findings in the various types of feces, we note from the figures and readings given in table 1, that the differences between a fermentative and a putrefactive flora are sufficiently striking, and therefore need little emphasis. The same cannot be said when we analyze the data characteristic of a normal stool. A correlation of the findings in normal with those in pathologic stools appears, therefore, to be of interest.

It is noted from table 1 that the fecal floras of an artificially fed child not suffering from any intestinal disturbances or the symptoms of a gastro-intestinal intoxication, resemble those of a slightly putrefactive stool. This fact is explained by the repeatedly made observation that infants fed on cow's milk have in the digestive tube a flora which is represented by a greater variety of bacteria than one commonly encountered in nurslings. Furthermore, the aciduric types of bacteria are suppressed and therefore an environment is created which

TABLE 1

PROTOTYPE OF STOOL SPECIMENS EXAMINED

	Normal Stool (12 specimens examined): Diet: Bovine milk. Character of Stools: Rather soft; light in color; odor normal			Putrefactive Stool (90 specimens examined): 3 types. Character of Stools: See text			Fermentative Stool (25 specimens examined): Character of Stools Usually light; yellow in color; foamy; acid odor		
	Direct Count of Smear (average count of 12 specimens examined): Percentage gram-negative organisms.....40.4% Percentage gram-positive organisms.....39.7% Percentage gram-positive rods.....59.6% Percentage gram-positive cocci.....40.4%			Direct Count of Smear (average count of 90 specimens examined): Percentage gram-negative organisms.....44.6% Percentage gram-positive organisms.....69.3% Percentage gram-positive rods.....40.2% Percentage gram-positive cocci.....62.3%			Direct Count of Smear (average count of 25 specimens examined): Percentage gram-negative organisms.....45.6% Percentage gram-positive organisms.....61.2% Percentage gram-positive rods.....70.4% Percentage gram-positive cocci.....30.8%		
	24 hours	48 hours	72 hours	24 hours	48 hours	72 hours	24 hours	48 hours	72 hours
Gelatin stab 22 C.	+	+	++	+	++	++++	—	—	—
Cresol-purple milk fermentation tube	9% gas Acid reaction Clot shrunken Whey expression +	No softening of clot	Peptonization +	4% gas Acid reaction Clot very soft No whey expression	Partial peptonization +	Partial peptonization ++	46% gas Acid reaction Clot very shrunken; Whey expression ++	No softening of clot	No peptonization
Fermentation tubes:									
1% glucose broth	22.2%	.....	.....	21.3%	.....	.....	22.2%	.....	.....
1% lactose broth	27.3%	.....	.....	23.2%	.....	.....	34.3%	.....	.....
1% saccharose broth	23.7%	.....	.....	9.9%	.....	.....	19.8%	.....	.....
Acetic acid glucose broth N/5	+	++	++	+	+	+	++	++	++
Acetic acid glucose broth N/10	++	++	++	+	+	+	++	++	++
Acetic acid glucose broth N/20	++	++	++	++	++	++	+++	+++	+++
Lactose agar spore plate	No growth —	No growth —	No growth —	Growth +	Growth +	Growth +	No growth —	No growth —	No growth —
Loeffler's serum slant 37 C.	Digestion — Discoloration —	Digestion + Discoloration +	Digestion + Discoloration +	Digestion + Discoloration +	Digestion ++ Discoloration ++	Digestion +++ Discoloration ++	Digestion — Discoloration —	Digestion — Discoloration —	Digestion — Discoloration +
Lactose agar plate anaerobic	564,600	.....	.....	784,400	.....	.....	621,820	.....	.....
Sugar-free plate aerobic	647,600	.....	.....	628 450	.....	.....	451 240	.....	.....
Lactose bile fermentation tube	37%	54%	.....	31%	46%	.....	55.5%	.....	.....
Endo plate aerobic 37 C.	Normal plate; acid reaction; growth ++			Proteolytic plate; alkaline reaction			Saccharolytic plate; acid reaction; growth +		
Blood milk fermentation tube anaerobic	Stormy fermentation			Stormy fermentation			No stormy fermentation		

is most favorable for the numerous proteolytic bacteria of infants' stools resulting from the feeding of cow's milk. The existence of these conditions is well indicated by the figures of the number of colonies recorded in the anaerobic and aerobic plates. The anaerobic bacterial count is approximately the same in all three types of infant floras; the aerobic count, which is due to B. coli, cocci and possibly a variety of spore-bearing rods, is noticeably reduced in a fermentative stool. In a normal stool this count is high and corresponds to the one noted in proteolytic specimens. The predominance of these aerobic

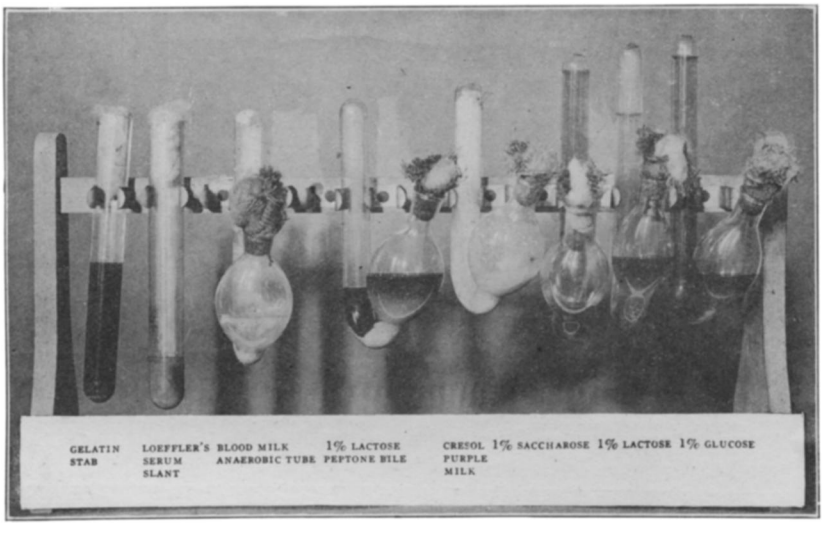

Fig. 1.—Main cultural reactions of a putrefactive fecal specimen.

organisms which have been introduced into the intestinal tract by the cows' milk, are the agents responsible for gelatin liquefaction; slight peptonization of the Loeffler's serum slant, and the partial softening of the milk curd.

Torrey[14] has been able to show quite recently, through carefully conducted experiments, that milk and a high casein diet stimulate a vigorous growth of saprophytic streptococci and enterococci. We made similar observations; the smears from the Loeffler's serum tubes — seeded with stool specimens derived from children fed on cows' milk — regularly demonstrated a predominance of streptococci. Also

[14] Jour. Med. Res., 1919, 39, p. 415.

Endo plates smeared with such stool emulsions revealed a majority of cocci which together with B. coli rendered the reaction of the plate distinctly acid.

In proceeding to the discussion of the putrefactive type of fecal flora, one is impressed with its similarity to the normal type of a cow's milk stool. There are only differences in degree, and it is sometimes exceedingly difficult to distinguish a semiputrefactive flora from a normal one. On the other hand, a putrefactive flora, which according to our conception may be responsible for clinical symptoms, is always

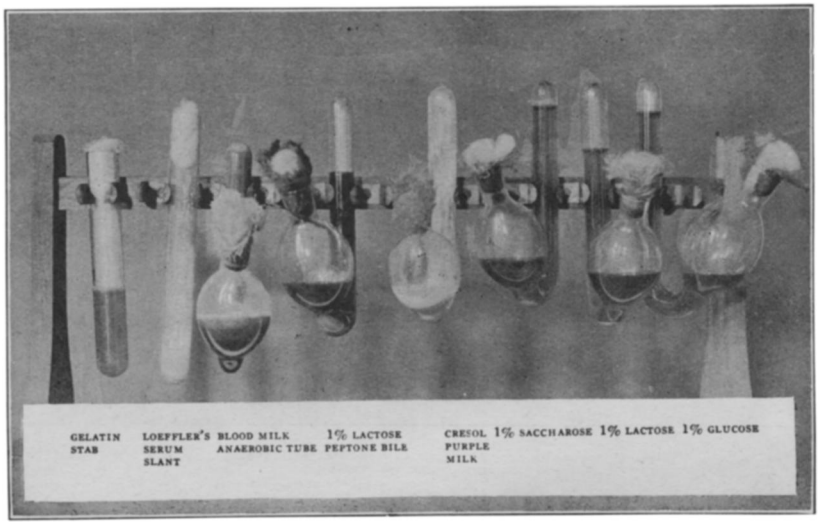

Fig. 2.—Main cultural reactions of a fermentative fecal specimen.

characterized by the following striking changes in the cultural tests: rapid liquefaction of the gelatin, the Loeffler's serum slant and the milk curd, as well as a strongly alkaline reaction of the Endo plates.

From a purely bacteriologic standpoint one is naturally interested in the question: Which bacterium or which group of organisms, or which "symbiotic complex" constitutes the poisonous elements of an obligate putrefactive flora? A number of chemists and experimental pathologists have repeatedly attempted to solve this complicated problem and several groups of organisms are thought to be responsible for the production of readily demonstrable toxic substances, like indol and phenol, in the intestinal content or the urine.

Herter[13] considers the B. coli and B. welchii, perhaps certain strains of B. proteus, of B. putrificus and of "B. malignant oedema"(?) either alone or in combination, responsible for intestinal putrefaction. The recent studies of Berthelot[15] and Rhein[16] on B. coli phenologenes and those of Van Loghem[17] and Groot[18] on the B. proteus anindologenes contribute considerable weight to the conclusions of Herter.

It is not unlikely that these various types become acclimatized to the digestive tubes of certain infants, act as obligate parasites and by their activities produce a putrefactive fecal flora (Jehle and Pincherle[19]). Naturally, this contention can only be proved by a systematic analysis of a large number of such stools and by a careful chemical study of the cleavage product of the isolated gram-negative rods on specially devised culture mediums.

The importance of B. welchii as a proteolytic organism, although functionally saccharolytic, calls for a revision of the published interpretations of the vast number of carefully collected facts, in the light of our more recent conception of the toxigenic properties of this bacteria and our growing knowledge concerning the difficulties of studying obligate anaerobes.

From personal experimentation we agree with Tenbroeck[20] that B. welchii undoubtedly plays only a very subordinate rôle in summer diarrhea. Recent observations have shown, however, that this organism may be responsible for some of the grave symptoms of intoxication in infantile dysentery. It is generally known that the accompanying flora of infantile dysentery is usually strongly putrefactive, containing an abnormal number of B. welchii (alkaline reaction of the stool). Following the dicta of Kendall, the pediatrist who is not especially trained in intestinal bacteriology is inclined to treat such cases by a strict lactose and carbohydrate regimen. The sudden shift in diet will naturally suppress the growth of the B. dysenteriae, but will also supply an excess of fermentable carbohydrates to the existing B. welchii and the other gas-producing intestinal bacteria. In case the strict lactose diet is continued for more than from 24 to 48 hours, in our experience grave symptoms of gas bacillus intoxications with sudden death are not uncommon.

[15] Ann. de l'Inst. Pasteur, 1918, 32, p. 17.
[16] Biochem. Ztschr., 1917, 84, p. 246.
[17] Ann. de l'Inst. Pasteur, 1918, 32, p. 295.
[18] Ann. de l'Inst. Pasteur, 1918, 32, p. 2.
[19] Wien. klin. Wchnschr., 1910, 23, p. 94.
[20] Bost. Med. and Surg. Jour., 1916, 174, p. 785.

How far this organism is responsible for a putrefactive flora we are not prepared to state definitely. As Ford, Blackfan and Batchelor[21] have stated, the mere presence of anaerobic, gram-positive rods is not indicative of anaerobic putrefaction, nor are these rods of diagnostic value since they are found in a variety of clinical conditions. The observations of Wollstein that the stools of infants contain more spores of B. welchii when kept on a protein than on a carbohydrate diet is fully confirmed in our study of several hundred stool specimens. We wish, furthermore, to emphasize the fact that a high carbohydrate diet has a striking inhibitive influence on B. welchii. According to Kendall and Day[22] and to the more recent work of Wolf and Harris,[23] B. welchii is fairly sensitive to a variety of acids, and cessation of growth occurs at P_H 4.82. It is therefore logical to produce an acid environment in the digestive tube of sufficient degree that multiplication of B. welchii is completely suppressed. The mere introduction of aciduric bacilli alone, as suggested by Kendall,[24] naturally will not bring about the desired result. In the absence of the proper pabulum of carbohydrates, the B. acidophilus can only lead a very limited intestinal existence, as has been emphasized repeatedly.

In only one or two of our patients with intestinal intoxication was the number of B. welchii spores so excessive that a sudden shift from a mixed diet to a pure carbohydrate one would have rendered the conditions for multiplication of this organism more favorable, and aggravated the clinical symptoms. The following principle was therefore applied: By means of starvation, if necessary by a cathartic and by feeding the child for from 24 to 48 hours on a strict protein diet, reduce the actual number of viable B. welchii, and then gradually substitute the pure proteid and buttermilk diet for one rich in carbohydrates. Progressively with the change of the putrefactive flora to a fermentative one, the number of spores decreases steadily until the methods we employed failed to demonstrate their presence. Again we offer this observation as a suggestion for further work on the rôle of the anaerobes in intestinal infections. In several instances we have isolated other anaerobes aside from B. welchii, and particularly in experimental work on dogs the frequent ubiquitous distribution of B. bifermentans and B. sporogenes along the large area of the digestive

[21] Am. Jour. Dis. Child., 1917, 14, p. 354.
[22] Boston Med. and Surg. Jour., 1912, 159, p. 754.
[23] Biochem. Jour., 1917, 11, p. 213-245.
[24] Am. Jour. Med. Sc., 1918 156, p. 157.

tract emphasized the fact that not every gram-positive sporulating gas-producing anaerobe is really a B. welchii.

The constant presence of strongly proteolytic gram-positive spore-bearing aerobes in the putrefactive stools has impressed us with the possible importance of this group of bacteria. The types thus far identified correspond with those described by Batchelor.[25] Torrey[14] considers these organisms of no significance from a biologic standpoint. We are not inclined to take such a radical view. It may be considered as having been proven through the studies of Ford, Blackfan and Batchelor[21] that these aerobic spore bearers are mechanically introduced with certain types of food, protein milk, farina, etc., richly infected with such bacteria. Changes in diet may, however, favor the growth, and under certain conditions a direct implantation in symbiosis with other bacteria may produce a suitable environment and render these fortuitously present micro-organisms the contributors of harmful cleavage products. It is our impression that a systematic inquiry into the various micro-organisms thus far accused of being responsible for intestinal intoxication is not complete without a consideration of the spore-bearing gram-positive aerobes.

The same suggestion can also be applied to the conception that B. putrificus is perhaps the organism responsible for intestinal putrefaction. The frequent occurrence of this bacterium, which has thus far been studied very superficially in putrefying protein material (Tulloch[26]) and also, for example, in pulp decay, is in many respects very suggestive. The description of two definite types of B. putrificus by Kligler[27] strongly emphasized the need of a more detailed study of this group of anaerobes. A possible adaptation of certain types to the intestines cannot be denied until further studies have been completed. And particularly the influence of aerobic bacteria on the growth of B. putrificus has to be considered. When viewed from this standpoint, the microbial associations of aerobic spore bearers in their relation to some of these anaerobes at least gain in importance as factors in a putrefactive fecal flora.

In turning to a discussion of the findings in a fermentative stool, some striking differences immediately attract our attention: complete absence of liquefaction of the gelatin and the Loeffler's serum slant, acid reaction of the Endo plate with a comparatively low count of the

[25] Jour. Bacteriol., 1919, 4, p. 23.
[26] Jour. Royal Army Med. Corps, 1917, 29, p. 631.
[27] Jour. of the Allied Dental Soc., 1915, 10, p. 321.

aerobic organisms. These cultural findings are the result of one established fact, namely, the predominance of aciduric bacilli, B. bifidus and B. acidophilus. An overwhelming presence of these organisms naturally has a depressing effect on the majority of saprophytic bacteria, which in turn produces an exceedingly simple type of flora. In contradistinction to the normal and putrefactive types of flora with a striking variety of bacterial species, the fermentative flora therefore consist of a few types only. The inhibitive effect of the acid metabolic split product of the aciduric organisms is particularly clearly shown in the disappearance of the fortuitously ingested streptococci and spore-bearing gram-positive rods. The latter statement applies not only to the aerobes, but to the anaerobes as well. The observations of Kendall explained that a high carbohydrate diet initiates through the metabolic activities of the aciduric organism such a low H-ion concentration of the intestinal content that B. welchii, for example, is unable to germinate. Therefore, it is not surprising that in our series of stool analyses stormy fermentation of the anaerobic milk tube was never observed. Clinical observation also supports our views that B. welchii and its supposedly harmful effects are more readily controlled by an eventually high carbohydrate than by a strict protein diet.

As is to be expected, not all of the stool specimens examined conform in their bacteriologic results to the three prototypes just discussed. Many reactions, sometimes rather puzzling, are obtained, and it is, therefore, considered necessary to present some of these results in table 2.

Two analyses of each of the three types are summarized. Fecal floras which differ from the recognized standards are designated as semiputrefactive and semifermentative, respectively. It is considered unnecessary to enter into a detailed discussion of their origin. We only desire to emphasize their occurrence and to impress the bacteriologist with the fact that only repeated bacteriologic examination of the feces by a uniform technic will sometimes establish the true nature of fecal flora.

A rapid and satisfactory diagnosis of the character of the fecal flora is sometimes possible by an abbreviated method. In cases of infantile dysentery the customary use of Endo or bromcresol-purple plates will, in conjunction with the isolation of the specific pathogenic dysentery bacilli, invariably suggest the character of the fecal flora. The detailed discussion given in previous paragraphs amply supports

TABLE 2

EXAMPLES OF PRONOUNCED AND MODIFIED TYPES OF FLORA

	Normal Stools						Putrefactive Stools						Fermentative Stools					
	Type, Normal 1			Type, Normal 2			Pronounced Putrefactive			Semiputrefactive			Pronounced Fermentative			Semifermentative		
	Name, Normal 8			Name, Normal 1			Name, Baby Cannon			Name, Baby Lugori			Name, Cogan			Name, Swartz		
	Character of Stool: Semiformed; light in color; odor normal			Character of Stool: Formed; color normal; odor bad			Character of Stool: Light in color; semiformed; spongy			Character of Stool: Formed; constipated; light in color			Character of Stool: Liquid stool; foamy; no odor			Character of Stool: Formed; dark in color; dry		
	Direct Count of Smear: Percentage gram-negative organisms, 61.9% Percentage gram-positive organisms, 38.1% Percentage gram-positive rods, 82.6% Percentage gram-positive cocci, 27.9% Date, Aug. 5, 1918			Direct Count of Smear: Percentage gram-negative organisms, 55.5% Percentage gram-positive organisms, 44.5% Percentage gram-positive rods, 53.6% Percentage gram-positive cocci, 46.4% Date, Aug. 3, 1918			Direct Count of Smear: Percentage gram-negative organisms, 38.9% Percentage gram-positive organisms, 61.7% Percentage gram-positive rods, 12.1% Percentage gram-positive cocci, 87.9% Date, Aug. 6, 1918			Direct Count of Smear: Percentage gram-negative organisms, 33.6% Percentage gram-positive organisms, 66.4% Percentage gram-positive rods, 42.6% Percentage gram-positive cocci, 57.4% Date, Sept. 19, 1918			Direct Count of Smear: Percentage gram-negative organisms, 45.8% Percentage gram-positive organisms, 54.2% Percentage gram-positive rods, 63.7% Percentage gram-positive cocci, 36.3% Date, July 23, 1918			Direct Count of Smear: Percentage gram-negative organisms, 15% Percentage gram-positive organisms, 85% Percentage gram-positive rods, 55% Percentage gram-positive cocci, 45% Date, Sept. 18, 1918		
	24 hours	48 hours	72 hours	24 hours	48 hours	72 hours	24 hours	48 hours	72 hours	24 hours	48 hours	72 hours	24 hours	48 hours	72 hours	24 hours	48 hours	72 hours
Gelatin stab 22 C.	+	+	++	—	—	+	++	+++	++++	+	++	+++	—	—	—	—	—	+
Cresol-purple milk fermentation tube	Acid reaction 7% gas clot semiformed whey +	Partial softening of clot	Peptonization +	Acid reaction 12% gas clot shrunken whey ++	No softening of clot	No peptonization	Acid reaction 5% gas clot soft whey —	Softening of clot	Peptonization ++	Acid reaction 10% gas clot semisoft whey +	Clot semisoft	Peptonization +	Acid reaction 16% gas clot very firm whey +++	No softening of clot	No peptonization	Acid reaction 40% gas clot firm whey ++	No softening of clot	No peptonization
Fermentation tubes:																		
1% Glucose broth	21%	....	....	21%	....	....	22%	....	....	46%	....	....	11%	....	....	15%	....	....
1% Lactose broth	22%	....	....	25%	....	....	37%	....	....	45%	....	....	30%	....	....	30%	....	....
1% Saccharose broth	28%	....	....	35%	....	....	28%	....	....	31%	....	....	9%	....	....	5%	....	....
Acetic acid glucose broth N/5	+	+	+	+	+	+	—	—	—	+	+	+	+	+	+	++	++	++
Acetic acid glucose broth N/10	++	++	++	++	++	++	+	+	—	+	+	+	++	++	++	++	++	++
Acetic acid glucose broth N/20	++	++	++	++	++	++	+	+	+	++	++	++	+++	+++	+++	+++	+++	+++
Lactose agar spore plate	No growth	No growth	No growth	No growth	No growth	No growth	Growth ++	Growth ++	Growth ++	No growth	No growth	No growth	No growth	No growth	No growth	No growth	No growth	No growth
Loeffler's serum slant 37 C.	Digestion — Discoloration —	Digestion — Discoloration —	Digestion + Discoloration +	Digestion — Discoloration —	Digestion + Discoloration +	Digestion + Discoloration +	Digestion + Discoloration ++	Digestion ++ Discoloration ++	Digestion +++ Discoloration ++	Digestion — Discoloration +	Digestion + Discoloration +	Digestion + Discoloration ++	Digestion — Discoloration —	Digestion — Discoloration —	Digestion — Discoloration —	Digestion — Discoloration —	Digestion — Discoloration —	Digestion + Discoloration +
Lactose agar plate anaerobic	369,600	.....	.....	990,000	.....	.....	812,000	.....	.....	1,632,000	.....	.....	660,000	.....	.....	865,000	.....	.....
Sugar-free plate aerobic	409,200	.....	.....	654,000	.....	.....	960,000	.....	.....	905,800	.....	.....	425,000	.....	.....	420,000	.....	.....
Lactose bile fermentation tube	30%	45%	.....	25%	40%	.....	30%	.....	.....	28%	35%	.....	40%	55%	.....	77%	.....	.....
Endo plate, aerobic 37 C.	Acid reaction; normal plate; growth ++			Acid reaction; normal plate; growth ++			Alkaline reaction; proteolytic plate			Alkaline reaction; semiproteolytic plate			Acid reaction; saccharolytic plate; growth +			Acid reaction; saccharolytic plate		
Blood milk fermentation tube anaerobic	No stormy fermentation			Stormy fermentation, B. welchii present			Stormy fermentation, B. welchii present			Stormy fermentation, B. welchii present			No stormy fermentation			No stormy fermentation		

this contention. For clinical use we have selected the gelatin stab tube, Loeffler's serum slant, cresol-purple milk with and without blood, 1 per cent. lactose peptone bile in fermentation tubes, and Endo plates. The results, which are characteristic for the various types of fecal floras, are self-explanatory and are shown in table 3.

REPEATED STOOL ANALYSES IN CHILDREN TREATED BY A STRICT CARBOHYDRATE DIET

It can be considered an established fact that the microbian flora of the intestinal tube stands in direct relationship to the diet of the host. Ingestion of even large numbers of bacteria foreign to the digestive tract does not itself seem to displace the common intestinal types in normal individuals. The studies of Herter[7, 8] and Kendall,[24] of Rettger and his pupils,[28, 29] and of Torrey[6, 14] demonstrate clearly this correlation between the types of bacteria found in the intestines and the clinical composition of the ingested food. The absence of carbohydrates in the diet produces a predominance of the proteolytic bacteria, which in turn may be responsible for putrefactive processes in the intestines, and by a continued absorption of the resulting toxic products are suspected by the clinician to be the main factors of some of the symptoms mentioned in the introduction.

The gratifying results obtained in the treatment of typhoid fever by a high carbohydrate diet, which through the painstaking studies of Torrey[6] can in part be explained by the development of a more favorable flora for the patient, immediately suggested to the pediatrist the use of a similar diet for the treatment of certain intestinal intoxication in children.

Originally, it was thought possible to apply the procedures of Torrey, as outlined in his work on the influence of a carbohydrate diet on the intestinal flora of typhoid patients, to the problem of the intestinal disorders of infants. The conditions of a private practice unfortunately prevented a careful quantitative control of the various elements of the diet ingested and systematic regular stool analyses were not possible. And again, the amounts and proportions of the carbohydrates fed were frequently changed, making it difficult to determine which substance exerted the most beneficial effect. The data

[28] Jour. Bacteriol., 1917, 2, p. 47.
[29] Centralbl. f. Bakteriol., I, 1914, 75, p. 219.

TABLE 3

ABBREVIATED FORM OF STOOL ANALYSIS

	Normal Stool			Putrefactive Stool			Fermentative Stool		
	24 hours	48 hours	72 hours	24 hours	48 hours	72 hours	24 hours	48 hours	72 hours
Gelatin tube	Liquefaction +	Liquefaction +	Liquefaction ++	Liquefaction ++	Liquefaction +++	Liquefaction +++	Liquefaction —	Liquefaction —	Liquefaction +
Loeffler's serum slant	Digestion and discoloration +	Digestion and discoloration +	Digestion and discoloration ++	Digestion and discoloration +	Digestion and discoloration +++	Digestion and discoloration ++++	Digestion* and discoloration —	Digestion and discoloration —	Digestion and discoloration +
Cresol-purple milk tube	Acid reaction Gas + Whey expression + Clot soft	Peptonization +	Peptonization +	Acid reaction Gas ± Whey expression + Clot soft	Peptonization ++	Peptonization ++	Acid reaction* Gas ++ Whey expression +++ Clot firm	Peptonization —	Peptonization —
1% lactose peptone bile	Gas production, 15 to 30% Smear of sediment: B. coli; gram-positive cocci; few gram-positive rods			Gas production, 10 to 25% Smear of sediment: B. coli; gram-positive cocci; many gram-positive rods			Gasproduction, 10 to 50% Smear of sediment: B. coli, few aciduric		
Endo plate	Reaction, acid Growth, ++ Odor, Acid Types of colonies, varied			Reaction, alkaline Growth, +++ Odor, pungent and offensive Types of colonies, Numberous			Reaction, acid Growth, + Odor, strongly acid Types of colonies, few		

* The interpretation of irregular reactions is found in paragraph (3) dealing with findings in Loeffler's serum medium slant and gelatin stab cultures (page 356)

TABLE 4

EFFECT OF A STRICT CARBOHYDRATE DIET ON PATIENTS WHOSE STOOLS HAVE BEEN DIAGNOSED PUTREFACTIVE

Name of patient	Jean Adams								
Character of stool	Semiformed; light; odor bad			Semiformed; no odor; foamy; moist			Formed; no odor; moist; light in color		
	First examination, May 29			Second examination, June 21 (22 days later)			Third examination, July 15 (24 days later)		
Direct count of smear: Percentage gram-negative organisms.. Percentage gram-positive organisms... Percentage gram-positive rods..... Percentage gram-positive cocci.....	25.1% 74.9% 54.1% 45.9%	Type of Flora: Putrefactive		11.3% 88.2% 71.5% 28.5%	Type of Flora: Semifermentative		8.0% 92.0% 87.4% 12.6%	Type of Flora: Fermentative	
	24 hours	48 hours	72 hours	24 hours	48 hours	72 hours	24 hours	48 hours	72 hours
Gelatin stab 22 C.	++	+++	++++	—	—	—	—	—	—
Cresol-purple milk fermentation tube...	Acid reaction 6% gas Clot soft Whey +	Clot soft Peptonization +	Peptonization ++	Acid reaction 14% gas Clot firm	No softening of clot	No peptonization	Acid reaction 5% gas Clot firm	No peptonization	No peptonization
Fermentation tubes: 1% glucose 1% lactose 1% saccharose	17% 12% 10%	....	....	5% 2% No gas	....	....	25% 54% 62%	....	....
Acetic acid glucose broth N/5..... Acetic acid glucose broth N/10..... Acetic acid glucose broth N/20.....	+ + ++	+ + ++	+ + ++	+ ++ +++	+ ++ +++	+ ++ +++	++ +++ +++	++ +++ +++	++ +++ +++
Lactose agar spore plate.....	No growth	No growth	No growth	No growth	No growth	No growth	No growth	No growth	No growth
Loeffler's serum slant 37 C.	Digestion ++ Discoloration —	Digestion +++ Discoloration +	Digestion +++ Discoloration ++	Digestion — Discoloration —	Digestion — Discoloration +	Digestion + Discoloration +	Digestion — Discoloration —	Digestion — Discoloration —	Digestion — Discoloration —
Lactose agar plate anaerobic.....	1,370,000	.....	.....	3,900,000	.....	.....	2,751,000	.....	.....
Sugar-free agar plate aerobic.....	330,000	.....	.....	520,000	.....	.....	456 000	.....	.....
Lactose bile fermentation tube.....	45% gas	.....	.....	20% gas	.....	.....	90% gas	.....	.....
Endo plate aerobic 37 C.	Alkaline reaction; proteolytic plate; growth +++	.....	.....	Acid reaction; saccharolytic plate; growth +	.....	.....	Acid reaction; saccharolytic plate; growth +	.....	.....
Blood milk fermentation tube anaerobic	Stormy fermentation	.....	.....	Stormy fermentation	.....	.....	Stormy fermentation	.....	.....

TABLE 4—*Continued*

EFFECT OF A STRICT CARBOHYDRATE DIET ON PATIENTS WHOSE STOOLS HAVE BEEN DIAGNOSED PUTREFACTIVE

Name of patient	Richard Hargreaves								
Character of stool	Liquid; greenish; undigested food			.....			Light; semiformed; odor not foul		
	First examination, July 18			Second examination, Aug. 15 (27 days later)			Third examination, Sept. 20 (36 days later)		
Direct count of smear:									
Percentage gram-negative organisms	68.1%	Type of Flora: Putrefactive		45.6%	Type of Flora: Semiputrefactive		42.6%	Type of Flora: Fermentative	
Percentage gram-positive organisms	31.9%			54.4%			57.4%		
Percentage gram-positive rods	26.2%			51.3%			67.8%		
Percentage gram-positive cocci	73.8%			48.7%			32.2%		
	24 hours	48 hours	72 hours	24 hours	48 hours	72 hours	24 hours	48 hours	72 hours
Gelatin stab 22 C.	++	+++	++++	+	++	++	—	—	—
Cresol-purple milk fermentation tube	10% gas Clot soft Whey +	Slight softening of clot	Peptonization ++	13% gas Clot semisoft filled with gas bubbles	No peptonization	No peptonization	— gas Clot shrunken When +++	No peptonization	No peptonization
Fermentation tubes:									
1% glucose	17%	.....	.....	30%	.....	.....	12%	.....	.....
1% lactose	65%	.....	.....	21%	.....	.....	20%	.....	.....
1% saccharose	34%	.....	.....	11%	.....	.....	18%	.....	.....
Acetic acid glucose broth N/5	—	—	—	++	++	++	++	++	++
Acetic acid glucose broth N/10	—	—	—	++	++	++	+++	+++	+++
Acetic acid glucose broth N/20	+	+	+	+++	+++	+++	+++	+++	+++
Lactose agar spore plate	Growth +	Growth ++	Growth ++	No growth	No growth	No growth	No growth	No growth	No growth
Loeffler's serum slant 37 C.	Digestion ++ Discoloration —	Digestion +++ Discoloration +	Digestion++++ Discoloration +	Digestion — Discoloration —	Digestion + Discoloration —	Digestion + Discoloration —	Digestion — Discoloration —	Digestion — Discoloration —	Digestion — Discoloration —
Lactose agar plate anaerobic	896,000	.....	.....	1,060,000	.....	.....	1,426 000	.....	.....
Sugar-free agar plate aerobic	1,200,000	.....	.....	1,958,000	.....	.....	1,634,000	.....	.....
Lactose bile fermentation tube	54%	67%	.....	37%	.....	.....	35%	42%	.....
Endo plate aerobic 37 C.	Alkaline reaction; proteolytic plate; growth +++	.....	.....	Acid reaction; slightly proteolytic plate; growth +	.....	.....	Acid reaction; normal plate; growth +	.....	.....
Blood fermentation tube anaerobic	Stormy fermentation	.....	.....	Stormy fermentation	.....	.....	Stormy fermentation	.....	.....

TABLE 4—*Continued*

EFFECT OF A STRICT CARBOHYDRATE DIET ON PATIENTS WHOSE STOOLS HAVE BEEN DIAGNOSED PUTREFACTIVE

Name of patient	Grace Holderman								
Character of stool	Semiformed; odor very bad; moist; dark			Formed; very dark in color; odor foul			Semiformed; light brown in color; odor		
	First examination, June 29			Second examination, Aug. 15 (46 days later)			Third examination, Dec. 2 (2 months later)		
Direct count of smear: Percentage gram-negative organisms Percentage gram-positive organisms Percentage gram-positive rods Percentage gram-positive cocci	42.6% 57.4% 61.2% 38 8%	Type of Flora: Putrefactive		32.8% 67.2% 81:5% 19.5%	Type of Flora: Semiputrefactive		33.5% 66.5% 76.2% 23 8%	Type of Flora: Semiputrefactive	
	24 hours	48 hours	72 hours	24 hours	48 hours	72 hours	24 hours	48 hours	72 hours
Gelatin stab 22 C.	++	+++	+++	+	++	++	+	+	++
Cresol-purple milk fermentation tube	Acid reaction 20% gas Clot soft Whey —	Clot softening Peptonization +	Peptonization ++	Acid reaction — gas Clot semiformed	No softening of clot	Peptonization ++	Acid reaction — gas Clot soft	No softening of clot	Peptonization +
Fermentation tubes: 1% glucose 1% lactose 1% saccharose	24% 20% 5%	....	....	20% 19% 20%	....	....	23% 18% 7%	....	....
Acetic acid glucose broth N/5 Acetic acid glucose broth N/10 Acetic acid glucose broth N/20	+ ++ +++	+ ++ +++	+ ++ +++	+ ++ +++	+ ++ +++	+ ++ +++	+ ++ +++	+ ++ +++	+ ++ +++
Lactose agar spore plate	Growth +	Growth ++	Growth ++	Growth +	Growth +	Growth +	No growth	No growth	No growth
Loeffler's serum slant 37 C.	Digestion + Discoloration —	Digestion ++ Discoloration +	Digestion ++ Discoloration ++	Digestion + Discoloration —	Digestion + Discoloration ++	Digestion ++ Discoloration ++	Digestion + Discoloration —	Digestion + Discoloration +	Digestion + Discoloration +
Lactose agar plate anaerobic	267,000	.....	.....	1,440 000	.....	.....	1.140 000	.....	.....
Sugar-free agar plate aerobic	237,800	.....	.....	2 625,000	.....	.....	1.230 000	.....	.....
Lactose bile fermentation tube	27%	.....	.....	68%	.....	.....	57%	.....	.....
Endo plate aerobic 37 C.	Alkaline reaction; proteolytic plate; growth +++	.....	.....	Slightly alkaline reaction; semiproteolytic plate; Growth ++	.....	.....	Slightly alkaline reaction; semiproteolytic plate; Growth ++	.....	.....
Blood fermentation tube anaerobic	Stormy fermentation	.....	.....	No stormy fermentation	.....	.....	No stormy fermentation	.....	.....

TABLE 4—*Continued*

EFFECT OF A STRICT CARBOHYDRATE DIET ON PATIENTS WHOSE STOOLS HAVE BEEN DIAGNOSED PUTREFACTIVE

Name of patient	Frederick Roeding								
Character of stool	Liquid; dark brown; odor bad			Formed; dry; dark brown in color			Formed; dark brown in color		
	First examination, July 18			Second examination, Aug. 11 (19 days later)			Third examination, Sept. 25 (33 days later)		
Direct count of smear: Percentage gram-negative organisms Percentage gram-positive organisms Percentage gram-positive rods Percentage gram-positive cocci	43.51% 56.49% 44.00% 66.00%	Type of Flora: Putrefactive		45.2% 54.8% 53.3% 46.7%	Type of Flora: Putrefactive		33.6% 66.4% 71.2% 28.8%	Type of Flora: Fermentative	
	24 hours	48 hours	72 hours	24 hours	48 hours	72 hours	24 hours	48 hours	72 hours
Gelatin stab 22 C.	++	++	+++	++	++	+++	—	—	—
Cresol-purple milk fermentation tube	Acid reaction 10% gas Clot soft Whey +	Softening of clot Peptonization +	Peptonization ++	Acid reaction 20% gas Clot soft	Softening of clot	Peptonization ++	Acid reaction 12% gas Clot firm Whey —	No softening of clot	No peptonization
Fermentation tubes: 1% glucose 1% lactose 1% saccharose	21% 22%	....	....	30% 36% 27%	....	....	15% 21% 13%	....	....
Acetic acid glucose broth N/5 Acetic acid glucose broth N/10 Acetic acid glucose broth N/20	+ + ++	+ + ++	+ + ++	+ ++ ++	+ ++ ++	+ ++ ++	+ ++ +++	+ ++ +++	+ ++ +++
Lactose agar spore plate	No growth	Growth ++	Growth ++	No growth	No growth	No growth	No growth	No growth	No growth
Loeffler's serum slant 37 C.	Digestion + Discoloration —	Digestion + Discoloration +	Digestion ++ Discoloration +	Digestion + Discoloration —	Digestion + Discoloration +	Digestion ++ Discoloration +	Digestion — Discoloration —	Digestion — Discoloration —	Digestion + Discoloration +
Lactose agar plate anaerobic	555,000	.....	.....	410,700	.....	.....	560 000	.....	.....
Sugar-free agar plate aerobic	400·000	.....	.....	422.000	.....	.....	412 000	.....	.....
Lactose bile fermentation tube	36%	.....	.....	47%	.....	.....	15%	.....	.....
Endo plate aerobic 37 C.	Alkaline reaction; proteolytic plate; growth +++	.....	.....	Semiproteolytic plate; alkaline reaction; Growth ++	.....	.....	Saccharolytic plate; acid reaction; Growth +	.....	.....
Blood fermentation tube anaerobic	Stormy fermentation	.....	.....	No stormy fermentation	.....	.....	No stormy fermentation	.....	.....

to be presented indicate therefore only a further application of the methods discussed, but they offer certain suggestions of considerable practical value.

A total of twelve cases was examined clinically by one of us (L. P.); stool specimens were forwarded to the laboratory when the child was first seen by the clinician. Subsequent fecal specimens were only analyzed when improvement in the clinical picture was definitely manifest, or when the child showed an unexplainable relapse. Inasmuch as most of the cases were not systematically investigated, we present here, in tables 4, 5, 6 and 7 only the summarized bacteriologic findings of four cases. The technical procedures and the interpretation of the findings are the same as already outlined. The treatment offered these cases, aside from a high calory carbohydrate diet, consisted of mild stimulation of the intestinal mobility to ensure the carbohydrates reaching the big bowel. Details concerning this phase are presented in the clinical paper of this series.

From all of the rather casually collected data one fact preeminently stands out; namely, the fecal flora of a child which shows the syndrome (tired look, dark circles around the eyes, liquid or constipated stools, anorexia, cyanosis, stupor and semiconsciousness, and laxity, recently described by one of us — L. P.) is always strongly putrefactive and rich in a variety of proteolytic bacteria. The actual number of aciduric organisms is always low, particularly in case 3, shown in table 6. In our experience such cases present a very unfavorable "facultative" intestinal flora, and changes in diet accomplish only slowly the desired increase in the aciduric bacilli. When a carbohydrate diet is prescribed for such cases, but the execution of the regimen is rather lax, relapses are common and in every instance the bacteriologic examinations showed a return from a semifermentative flora to a strongly putrefactive one. On the other hand, a clinical improvement or even a complete cure from an apparently chronic intoxication by the use of a strict carbohydrate diet was, in our examinations, always strikingly indicated by a fermentative flora. The increase of aciduric bacilli over the number noted at the first examination was always very marked; in some cases this group of organisms outnumbered the other viable bacteria about 15 or 25 to 1. The cultural results on the anaerobic lactose agar plate, the acetic acid glucose broth tubes, and the direct counts presented in the tables give sufficient evidence to that effect. Therefore, the results thus far collected prove

that the fecal flora of a child's intestinal tract may be changed through a strict, liberal carbohydrate diet to such a degree that it reverts toward, or completely to, the fermentative non-gas-producing flora of the nursling.

In this connection it may be mentioned that individual differences play an important rôle. Torrey in dogs, and Hull and Rettger[29] in rat experiments observed that changes were more readily effected in some individuals than in others. In our experience clinical recovery from these intestinal intoxications can only be associated with a complete transformation of the putrefractive-proteolytic type of stool to a decidedly fermentative one. Such a result is usually only accomplished after a prolonged, strict carbohydrate diet regimen. The mere elimination of the obligate putrefactive organisms and a moderate development of the aciduric types is not sufficient. This particular fact explains in part the long time interval which as a rule was necessary to transform the initial strongly putrefactive flora. If, however, the initial flora was more favorable, semiputrefactive only in character, a change through a carbohydrate diet can be readily achieved in from 2 to 6 weeks.

It is generally stated from experiments on laboratory animals that a transformation of the fecal flora through carbohydrate diet may be accomplished in a few days. In our experience with children with an unfavorable initial flora from 10 to 40 days are, as a rule, required for this transformation. Aside from the individual differences of the initial flora of the patients already discussed, the number and variety of types of organisms and the length of the infants' intestinal tubes exceed those of many lower animals. It is therefore not surprising that the time element to produce a complete transformation must be taken into account from a therapeutic point of view. In fact, our observations on children are well supported by the data which Torrey has collected from the examinations of adult cases: Even in those patients with an initial favorable "facultative" intestinal flora, at least two weeks were required to increase through a carbohydrate diet the B. acidophilus to a level characteristic of an obligate fermentative flora.

As already stated, we were unable to make systematic examinations of the feces at regular time intervals. We therefore cannot say how soon the obligate putrefective organisms were suppressed, after the strict regimen of a carbohydrate diet had been instituted. We know from the case histories that sometimes several weeks elapsed before clinical improvement was noticeable. Judging from this and from the

fact that improvement is not possible without a definite transformation to a predominant aciduric simplified flora, we also feel justified in concluding that a change in flora does not occur inside of a few days. The transformed flora persists as long as the special diet is continued. Only under a diet with animal proteins eliminated and with a predominant carbohydrate foundation are the results more than transitory.

Some observations also have shown that intercurrent and possibly focal infections like tonsillitis or rhinitis have an "inhibitive' 'influence on the normally progressing transformation of a harmful putrefactive flora. Tonsillectomy was associated, in a few instances, with a remarkably rapid change of flora, which, in spite of a strict carbohydrate diet enforced by feeding of B. acidophilus and other therapeutic measures, had remained stationary for several months. The various factors responsible for this influence can only be surmised until further studies have been completed, and are therefore reserved for another publication.

The main result of this study of selected cases under treatment is, in our experience, shown by the reliable information and the diagnostic value which detailed stool analyses offer to the clinician. The application of the procedures outlined to a variety of infants' diseases may furnish data in the future which, together with the clinical observations, will help to unravel the manifold factors of so-called gastro-intestinal intoxication in infancy.

In this connection the next most important question is, Which foodstuffs exert the strongest transforming influence on the intestinal flora? Most of the workers in this field of applied bacteriology agree that the feeding of such carbohydrate foods which on hydrolysis readily yield dextrose, as for example lactose, are most suitable. Torrey[14] has shown that in adult typhoid fever cases kept on a regimen of 250 gm. and upwaŕd of lactose, there resulted a transformation of the ordinary type of flora to one strongly dominated by B. acidophilus. On the other hand, Sittler[2] demonstrated that the feeding of carbohydrates which on hydrolysis yield levulose (cane sugar) tend to bring about a mixed flora with an overwhelming number of B. welchii. Maltose, lactose and dextrose are the substances most suitable for the control of proteolytic organisms of the digestive tube. Experiments on children and dogs, with different foodstuffs, were in progress when quite recently our studies along these lines were materially enlightened by the excellent study of Torrey. By experiments on dogs he was able to show that lactose and dextrin added to the diet completely suppress

the proteolytic types of fecal bacteria, even including B. coli commonly found in the dog's intestinal tract. Starchy foods (white bread, potatoes and beans) also tended to effect a simplification of the intestinal flora.

Among the proteins responsible for intestinal putrefaction, milk casein exhibited the tendency far less markedly than did meat protein. Vegetable proteins also fail to offer encouragement to the growth of proteolytic organisms. We have confirmed these observations in a few feeding experiments on dogs and we await with great interest the application of this knowledge to the treatment of children showing intestinal intoxications. We have thus far gained the impression that in the children's digestive tube the effect of cow's milk proteins is almost, if not quite, as conducive to the growth of proteolytic bacteria as is meat protein in the dog's intestinal tract.

Children with an unfavorable initial flora respond very slowly and incompletely to the transforming influence of a carbohydrate diet, as already discussed in detail. In such cases it may be advisable to implant aciduric organisms by feeding pure culture or tablets of B. acidophilus. It is not unlikely that the application of the so-called "broma therapy" of Kendall, the intestinal implantation of the beneficial B. acidophilus, should be combined with the carbohydrate diet and made a routine procedure in all intestinal, or even in acute, infections of childhood (Kendall). In a few cases of infantile dysentery we have seen excellent results by the use of this principle. Until more cases have been studied bacteriologically, we offer the above considerations merely as suggestions to the clinician.

SUMMARY

This article contains the description and interpretation of a bacteriologic method applicable to the examination of fecal specimens of children apparently suffering from "intestinal intoxication." It is furthermore shown that a strongly putrefactive flora is associated with certain groups of intestinal disorders of infancy, and that clinical improvement is practically always accomplished by a strict carbohydrate diet. The progress in the transformation of the intestinal flora is readily controlled by the cultural tests described.[30]

[30] In addition to the references given, the following may be of interest:
Moro: Johr. f. Kinderh., 1905, 61, pp. 687 and 870.
Rettger and Horton: Centralbl. f. Bakteriol., 1914, 73, p. 362.
Tissier: Recherches sur le flore intestinale des nourrissons, These de Paris, 1900.
Tissier: Ann. de l'Inst. Pasteur. 1905, 19, p. 109.
Wollstein: Am. Jour. Child., 1912, 4, 279.